ISSN 1070-3632, Russian Journal of Bioorganic Chemistry, 2023, Vol. 49, No. 5, pp. 970–975. © Pleiades Publishing, Ltd., 2023.

Coumarin Hybrids as Cholinesterase Inhibitors

M. Yildirim[a,*], M. Ersatır[b], S. Yalin[c], and E. S. Giray[b]

[a] *Department of Pharmacy Services, Vocational School of Health Services, Tarsus University, Mersin, 33400 Turkey*
[b] *Department of Chemistry, Arts and Science Faculty, Cukurova University, Adana, 01330 Turkey*
[c] *Department of Biochemistry, Pharmacy Faculty, Mersin University, Mersin, 33110 Turkey*
**e-mail: metinyildirim4@gmail.com*

Received January 2, 2023; revised January 13, 2023; accepted January 14, 2023

Abstract—Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are two important enzymes involved in the breakdown of the neurotransmitter acetylcholine and other choline esters. Coumarins are biologically active compounds naturally found in the fruit, bark, stems, and leaves of many plants, including Tonka bean, acacia, lavender, deer tongue, apricot, strawberry, cherry, and cinnamon. Coumarin and coumarin hybrids have antitumor, antioxidant, antiviral, anticonvulsant, anti-inflammatory, antimicrobial, and antiproliferative activity. AChE and BuChE enzyme inhibition effects of 12 different coumarin derivatives (**Ia–Ih**) and (**IIa–IId**) synthesized and 16 coumarin-selenophene hybrid compounds (**IVa–IVh**) and (**Va–Vh**) were investigated. Compound (**Vc**) and (**Vg**) showed the most potent inhibitor activity against AChE.

Keywords: AChE, BuChE, coumarin, coumarin-selenophene, alzheimer's disease

DOI: 10.1134/S1068162023050035

INTRODUCTION

Alzheimer's disease is the most prevalent form of dementia, a progressive, degenerative disorder that destroys brain cells and causes memory, cognitive, and behavioral impairments [1]. Alzheimer's is a debilitating and ultimately fatal condition that currently has no cure. The most common symptom of Alzheimer's is memory loss. This can include difficulty remembering things that have recently happened, difficulties with problem-solving, and difficulty with language [2]. As the disease progresses, these symptoms can worsen and additional symptoms can appear, such as confusion, disorientation, difficulty with everyday tasks, mood swings, and changes in behavior [3]. There are a number of drugs known so far that are used for about 6–12 months to improve symptoms. In the USA, the number of people suffering from Alzheimer's is expected to increase exponentially and reach 13,5 million by 2050 [4]. In recent years, a number of potential disease-controlling Alzheimer's drugs have been evaluated through clinical trials, and many drug trials are ongoing. However, no new drug that has not been approved by the FDA has entered the literature since 2003 [5].

Acetylcholinesterase (AChE) is an enzyme found in the brain, peripheral nervous system, and neuromuscular junction that catalyzes the breakdown of acetylcholine into choline and acetic acid. AChE is essential for the normal functioning of the nervous system, as it prevents overstimulation of the nerve cells and ensures that the signals they send are properly regulated [6]. AChE is inhibited by a number of agents, such as organophosphates, which cause the accumulation of acetylcholine in the nervous system and can lead to symptoms of intoxication [7]. Several AChE inhibitor drugs are available in the market, such as donepezil [8], galantamine [9], memantine [10], and tacrine [11].

Butyrylcholinesterase (BuChE) is an enzyme found in the brain, peripheral nervous system, and neuromuscular junction that hydrolyzes choline esters, such as butyrylcholine and acetylcholine. BuChE is an important and vital role in nerve impulse transmission [12].

Coumarins are biologically active compounds naturally found in the fruit, bark, stems and leaves of many plants, including Tonka bean, acacia, lavender, deer tongue, apricot, strawberry, cherry, and cinnamon. Coumarin and coumarin hybrids have antitumor [13], antioxidant [14, 15],

Abbreviations: AChE, acetylcholinesterase; BuChE, butyrylcholinesterase.

Table 1. AChE and BuChE inhibitor activity of 3-aceltycoumarins, coumarin-selenophene hybrids, 4-methylcoumarins, selenophenes, and carbostyril-selenophenes (**Ia–Vh**)

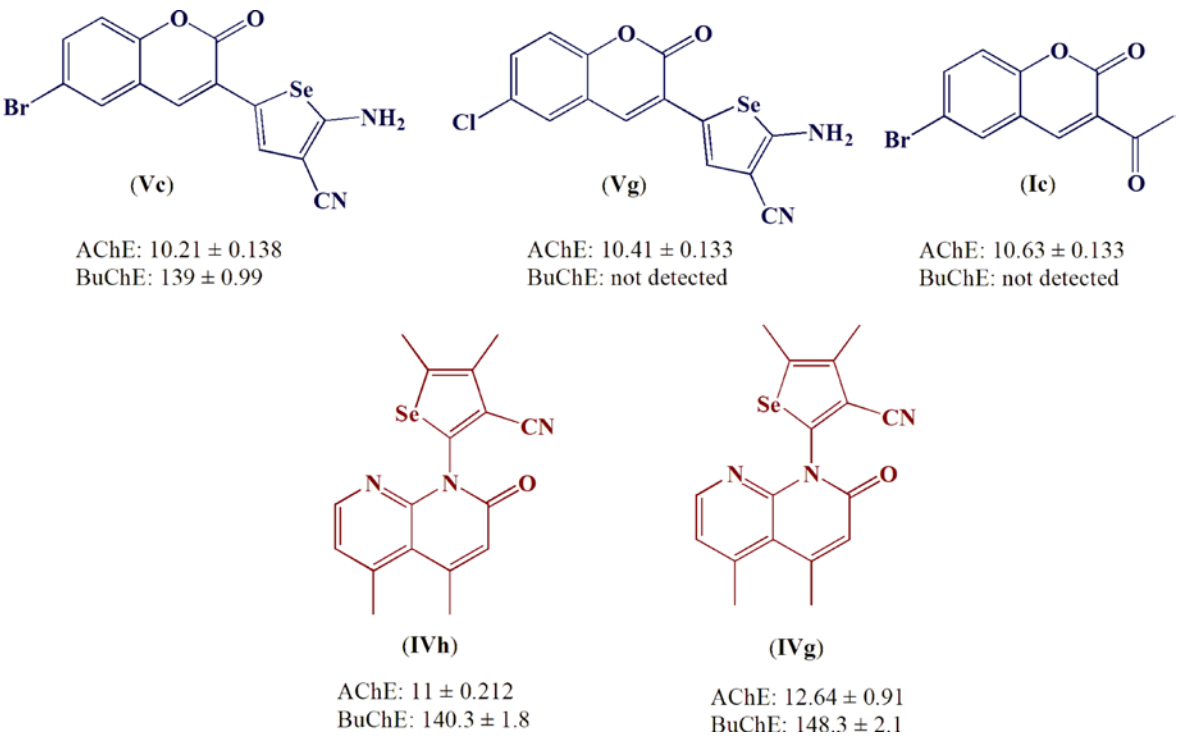
Entry	Compound	R	R_2	R_3	R_4	X	AChE IC_{50} (μM)	BuChE IC_{50} (μM)
3-Acetylcoumarins								
1	(**Ia**)	H	–	–	–	–	15.08 ± 0.32	Nd
2	(**Ib**)	7-OCH_3	–	–	–	–	15.13 ± 0.2	Nd
3	(**Ic**)	6-Br	–	–	–	–	10.63 ± 0.133	133 ± 1.03
4	(**Id**)	7-OH	–	–	–	–	24 ± 0.221	Nd
5	(**Ie**)	6-NO_2	–	–	–	–	22 ± 0.131	Nd
6	(**If**)	6,8-Br	–	–	–	–	22.3 ± 0.09	Nd
7	(**Ig**)	6-Cl	–	–	–	–	15.05 ± 0.11	141 ± 1.12
8	(**Ih**)	7-$N(C_2H_5)_2$	–	–	–	–	63 ± 1.3	Nd
Coumarin-selenophene hybrids								
9	(**Va**)	H	–	–	–	–	15.15 ± 0.21	Nd
10	(**Vb**)	7-OCH_3	–	–	–	–	15.01 ± 0.66	Nd
11	(**Vc**)	6-Br	–	–	–	–	10.21 ± 0.138	139 ± 0.99
12	(**Vd**)	7-OH	–	–	–	–	18.94 ± 0.21	Nd
13	(**Ve**)	6-NO_2	–	–	–	–	18.3 ± 0.44	Nd
14	(**Vf**)	6,8-Br	–	–	–	–	20 ± 0.212	Nd
15	(**Vg**)	6-Cl	–	–	–	–	10.41 ± 0.133	Nd
16	(**Vh**)	7-$N(C_2H_5)_2$	–	–	–	–	24.18 ± 0.135	Nd
4-Methylcoumarins								
17	(**IIa**)	–	OH	H	–	CH	18.2 ± 0.919	Nd
18	(**IIb**)	–	CH_3	H	–	CH	18 ± 1.21	Nd
19	(**IIc**)	–	H	H	–	N	17.9 ± 0.48	Nd
20	(**IId**)	–	H	CH_3	–	N	16.39 ± 0.31	Nd
Selenophenes								
21	(**IIIa**)	–	–	–	H	–	19.7 ± 1.09	Nd
22	(**IIIb**)	–	–	–	CH_3	–	19.3 ± 0.88	Nd
Carbostyril-selenophenes								
23	(**IVa**)	–	OH	H	H	CH	14.53 ± 0.45	Nd
24	(**IVb**)	–	OH	H	CH_3	CH	14.01 ± 0.22	Nd
25	(**IVc**)	–	CH_3	H	H	CH	13.39 ± 0.33	Nd
26	(**IVd**)	–	CH_3	H	CH_3	CH	13 ± 0.02	Nd
27	(**IVe**)	–	H	H	H	N	14.41 ± 0.32	Nd
28	(**IVf**)	–	H	H	CH_3	N	13.56 ± 0.41	Nd
29	(**IVg**)	–	H	CH_3	H	N	12.64 ± 0.91	148.3 ± 2.1
30	(**IVh**)	–	H	CH_3	CH_3	N	11 ± 0.212	140.3 ± 1.8
Reference substance								
31	Tacrin	–	–	–	–	–	41	16.3

antiviral [16, 17] , anticonvulsant [18], anti-inflammatory [19, 20], antimicrobial [21, 23], and antiproliferative activity [14, 15]. It has been shown that hybrid compounds formed by combining coumarin compounds with various molecules exhibited increased biological activity [14].

In this study, acetylcholinesterase and butyrylcholinesterase enzyme inhibition effects of 12 different coumarin derivatives (**Ia–Ih**) and (**IIa–IId**) synthesized and 16 coumarin-selenophene hybrid compounds (**IVa–IVh**) and (**Va–Vh**) were investigated.

RESULTS AND DISCUSSION

The mechanism and treatment methods of Alzheimer's, which is called the disease of our age, are still not fully determined. Therefore, studies on the synthesis and



Scheme 1. The most active compounds.

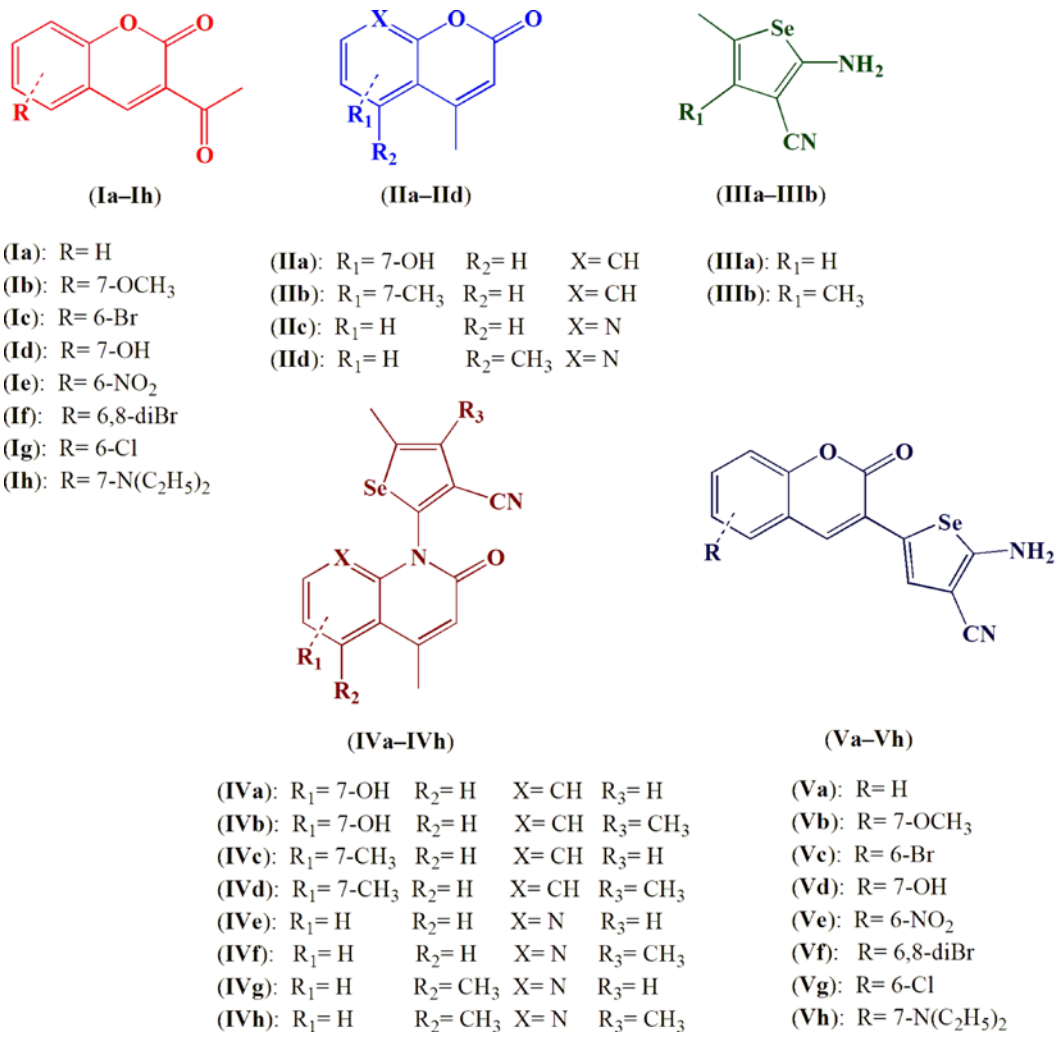
development of cholinesterase inhibitors are important. For this purpose, AChE and BuChE enzyme inhibition activities of synthesized hybrid compounds and selenophene and coumarin compounds used as intermediates in the synthesis of these compounds were tested.

AChE enzyme inhibitor activity of 3-acetylcoumarin derivatives (**Ia–Ih**) obtained by Knoevenagel condensation reaction are in the range of IC_{50} = 10.63 ± 0.13 – 63 ± 1. Among these compounds, the highest activity was obtained in compound (**Ic**) with –Br substituent at C-6 in the coumarin ring. (IC_{50} = 10.63 ± 0.13; Table 1). In case of –Cl substituent at C-6, IC_{50} = 15.05 ± 0.11. In the presence of substituents such as –OH and $–N(C_2H_5)_2$ at C-7, the IC_{50} values were the lowest among the coumarins (24 ± 0.22 and 63 ± 1, respectively).

AChE inhibitor activity of 4-methylcoumarin compounds (**IIa–d**) synthesized by the Pechmann reaction are better than 3-acetylcoumarins (in the range of IC_{50} = 16.39 ± 0.31–18.2 ± 0.92). Among these compounds, pyridine derivatives (**IIc**) and (**IId**) show better activity than benzene derivatives (**IIa**) and (**IIb**). The AChE inhibition of selenophene derivatives obtained by the Gewald reaction (**IIIa–IIIb**) was similar (IC_{50} = 19.3 ± 0.88 and 19.7 ± 1.10). Among these compounds, the presence of $–CH_3$ group on R_4 in the selenophene ring showed better activity than the –H substituent on the same carbon.

AChE inhibition values of coumarin-selenophene hybrid compounds synthesized from 3-acetylcoumarin (**Va–Vh**) are in the range of IC_{50} = 10.21 ± 0.14–24.18 ± 0.14. Among the 3-acetylcoumarin compounds, (**Ic**) and (**Ig**) showed the most potent inhibitor activity. Moreover, their hybrids form (**Vc**) and (**Vg**) were also exhibited as the most active compounds. In addition, compound (**Vc**) has the best inhibition value among all tested compounds against AChE (IC_{50} = 10.21 ± 0.14). 1h compound, which is the least active among the 3-acetylcoumarin compounds, was synthesized the coumarin-selenophene hybrid, a significant increase in AChE activity was determined (Table 1).

New carbostyryl-selenophene hybrid compounds (**IVa–IVh**) were obtained by reacting 4-methylcoumarin derivatives obtained by Pechmann reaction with selenophene compounds obtained by Gewald reaction. AChE inhibition values of these compounds are examined, it can be said that they show a much better inhibition effect than their constituents coumarin and selenophene (IC_{50} = 11 ± 0.21–14.53 ± 0.45). Among these hybrids, (**IVg**) and (**IVh**) hybrids obtained by the reaction of (**IId**) coumarin compound with (**IIIa**) and (**IIIb**) selenophene derivatives showed the best activity (Table 1). Most active compounds are shown in Scheme 1. In this study, the BuChE inhibitory effect of the synthesized molecules



Scheme 2. AChE and BuChE inhibition activity of tested compounds.

was also investigated. However, the BuChE inhibitory activity of these molecules was found to be lower or not active at all than Tacrin (IC_{50} = 16.3 μM).

EXPERIMENTAL

In this study, AChE and BuChE inhibition activities of 12 coumarin derivatives (**Ia–Ih**) and (**IIa–IId**), 2-selenophene derivatives (**IIIa–IIIb**) and 16 coumarin-selenophene hybrid compounds (**IVa–IVh**) and (**Va–Vh**) were investigated (Scheme 2). The structures of all synthesized compounds were elucidated by FT-IR, ^{1}H NMR and ^{13}C NMR methods. Spectral data of compounds are given in the supplementary information.

AChE/BChE enzyme activity. AChE and BuChE enzyme were purchased from Sigma-Aldrich. Cholinesterases catalyze the breakdown reaction of acetylcholine to thiocholine and acetate. The principle of the study is based on measuring the absorption of the reaction product at 412 nm with a spectrophotometer [24].

CONCLUSIONS

AChE and BuChE enzyme inhibition tests of coumarin, selenophene and coumarin-selenophene hybrid compounds were performed. Active coumarin or coumarin hybrid compounds were synthesized, the presence of substituents such as –Br, –Cl at C-6 increased AChE inhibition. In the presence of substituents such as –OH and –$N(C_2H_5)_2$ at C-7, the AChE inhibition of the compound was reduced. Synthesis of 4-methylcoumarin derivatives to be synthesized by Pechmann reaction with pyridine derivatives containing –OH groups increased inhibition. Among coumarins, 3-acetylcoumarin derivatives showed better AChE inhibition than Pechmann coumarin derivatives. In 3-acetylcoumarin-selenophene hybrid

compounds, AChE inhibition is increased in the presence of –Br and –Cl substituents at C-6 in the coumarin ring. In the case of R_4 methyl substituent in selenophene derivatives, a more active compound is obtained than if it is hydrogen. Among all compounds, carbostyryl-selenophene hybrid compounds showed better AChE inhibition activity. In general, hybrid compounds are more active than the starting compounds. It is possible to obtain more active compounds with new research on carbostyryl-selenophene derivatives. In future studies, it may be possible to use especially Coumarin-selenophene hybrids (**Vc**) and (**Vg**) and carbostyryl-selenophene derivatives (**IVa–IVh**) in Alzheimer's research and to modify these compounds.

FUNDING

This work was supported by regular institutional funding, and no additional grants were obtained.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

Informed consent was not required for this article.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTIONS

Authors MY, ME, and ESG designed the experiments; authors ME and ESG synthesized the samples. Authors ME and ESG carried out studies using NMR spectroscopy. Authors MY and SY made enzyme inhibition studies. Authors MY and ME contributed to manuscript preparation. All authors participated in the discussions

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1068162023050035

REFERENCES

1. Knopman, D.S., Amieva, H., Petersen, R.C., Chetelat, G., Holtzman, D.M., Hyman, B.T., Nixon, R.A., and Jones, D.T., *Nat. Rev. Dis. Primers*, 2021, vol. 7, p. 33. https://doi.org/10.1038/s41572-021-00269-y
2. Kumar, A., Singh, A., and Ekavali., *Pharmacol. Rep.*, 2015, vol. 67, pp. 195–203. https://doi.org/10.1016/j.pharep.2014.09.004
3. Long, J.M. and Holtzman, D.M., *Cell*, 2019, vol. 179, pp. 312–339. https://doi.org/10.1016/j.cell.2019.09.001
4. Barnes, L.L., *Nat. Rev. Neurol.,* 2022, vol. 18, pp. 56–62. https://doi.org/10.1038/s41582-021-00589-3
5. Cummings, J.L., Morstorf, T., and Zhong, K., *Alzheimers Res. Ther.*, 2014, vol. 6, p. 37. https://doi.org/10.1186/alzrt269
6. Trang, A. and Khandhar, P.B., In *StatPearls*, Treasure Island (FL), 2022.
7. Guo, J.X., Wu, J.J., Wright, J.B., and Lushington, G.H., *Chem. Res. Toxicol.*, 2006, vol. 19, pp. 209–216. https://doi.org/10.1021/tx050090r
8. Silva, M.A., Kiametis, A.S., and Treptow, W., *J. Chem. Inf. Model*, 2020, vol. 60, pp. 3463–3471. https://doi.org/10.1021/acs.jcim.9b01073
9. Scott, L.J. and Goa, K.L., *Drugs*, 2000, vol. 60, pp. 1095–1122. https://doi.org/10.2165/00003495-200060050-00008
10. Saeedi, M. and Mehranfar, F., *Recent Pat. Biotechnol.*, 2022, vol. 16, pp. 102–121. https://doi.org/10.2174/1872208316666220302115901
11. Sivaraman, B., Raji, V., Velmurugan, B.A., and Natarajan, R., *CNS Neurol. Disord. Drug Targets*, 2022, vol. 21, pp. 427–449. https://doi.org/10.2174/1871527320666210928160159
12. Lockridge, O., *Pharmacol. Ther.*, 2015, vol. 148, pp. 34–46. https://doi.org/10.1016/j.pharmthera.2014.11.011
13. Sandhu, S., Bansal, Y., Silakari, O., and Bansal, G., *Bioorg. Med. Chem.*, 2014, vol. 22, pp. 3806–3814. https://doi.org/10.1016/j.bmc.2014.05.032
14. Ersatir, M., Yildirim, M., Giray, E.S., and Yalin, S., *Monatsh. Chem.*, 2020, vol. 151, pp. 625–636. https://doi.org/10.1007/s00706-020-02573-x
15. Ersatir, M., Yildirim, M., and Giray, E.S., *Synthetic. Commun.*, 2021, vol. 51, pp. 290–301. https://doi.org/10.1080/00397911.2020.1825744
16. Curini, M., Epifano, F., Maltese, F., Marcotullio, M.C., Gonzales, S.P., and Rodriguez, J.C., *Aust. J. Chem.*, 2003, vol. 56, pp. 59–60. https://doi.org/10.1071/Ch02177
17. Hassan, M.Z., Osman, H., Ali, M.A., and Ahsan, M.J., *Eur. J. Med. Chem.*, 2016, vol. 123, pp. 236–255. https://doi.org/10.1016/j.ejmech.2016.07.056
18. Siddiqui, N., Arshad, M.F., and Khan, S.A., *Acta Pol. Pharm.*, 2009, vol. 66, pp. 161–167.

19. Tapanyigit, O., Demirkol, O., Guler, E., Ersatir, M., Cam, M.E., and Giray, E.S., *Arab. J. Chem.*, 2020, vol. 13, pp. 9105–9117. https://doi.org/10.1016/j.arabjc.2020.10.034
20. Yildirim, M., Unal, Z.N., Ersatir, M., Yetkin, D., Degirmenci, U., and Giray, E.S. *Russ. J. Bioorg. Chem.*, 2022, vol. 48, pp. 1209–1214. https://doi.org/10.1134/S1068162022060279
21. Reddy, K.R., Mamatha, R., Babu, M.S.S., Shiva Kumar, K., and Jayaveera, K.N., *J. Heterocyclic Chem.*, 2014, vol. 51, pp. 132–137. https://doi.org/10.1002/jhet.1745
22. Thakrar, S., Bavishi, A., Radadiya, A., Vala, H., Parekh, S., Bhavsar, D., Chaniyara, R., and Shah, A., *J. Heterocyclic Chem.*, 2014, vol. 51, pp. 555–561. https://doi.org/10.1002/jhet.1641
23. Yildirim, M., Poyraz, S., and Ersatir, M., *Med. Chem. Res*., 2023, vol. 32, pp. 1–26. https://doi.org/10.1007/s00044-023-03025-x
24. Ellman, G.L., Courtney, K.D., Andres, V., Jr., and Feather-Stone, R.M., *Biochem. Pharmacol.*, 1961, vol. 7, pp. 88–95. https://doi.org/10.1016/0006-2952(61)90145-9